Int. J. Devl Neuroscience 30 (2012) 427–433

Contents lists available at SciVerse ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Neurodevelopmental anomalies of the hippocampus in rats exposed to weak intensity complex magnetic fields throughout gestation

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ARTICLE INFO

Article history:
Received 26 February 2011
Received in revised form 21 July 2012
Accepted 22 July 2012

Keywords:
Magnetic field
Prenatal development
Hippocampus
Fear conditioning
Teratogenic
Rat

ABSTRACT

There has been increasing interest on the possible harmful effects of prenatal exposure to magnetic fields. To investigate the effect of weak intensity magnetic fields on the prenatal brain, pregnant Wistar rats were continuously exposed to one of four intensities (reference: 5–20 nT; low 30–50 nT; medium 90–580 nT; high 590–1200 nT) of a complex magnetic field sequence designed to interfere with brain development. As adults, rats exposed to the low-intensity (30–50 nT) complex magnetic field displayed impairments in contextual fear learning and showed anomalies in the cytological and morphological development of the hippocampus. In particular, low-intensity exposures resulted in a reduction in overall hippocampal size and promoted subtle dysgenesis of the CA1 and CA3 regions. In contrast, exposure to weaker or stronger intensities of the same complex magnetic field pattern did not interfere with hippocampal development or fear behavior. These findings suggest that prenatal exposure to complex magnetic fields of a narrow intensity window during development can result in subtle but permanent alterations in hippocampal microstructure and function that can have lasting effects on behavior.

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1. Introduction

There is considerable evidence that exposure to time-varying magnetic fields, even those of very low-intensity (<10 mT), can affect living organisms and their behavior (see (Juutilainen, 2005; Lovely, 1988; Nordeon and Ramel, 1992; Persinger et al., 1973). One of the most important and controversial effects concerns the action of weak magnetic fields on cells or cellular systems that are in a transitional state, such as those characteristic of embryonic and foetal periods (Mcleod et al., 1992). Several studies have shown that pulsed or sinusoidal magnetic fields can produce embryological anomalies in chicken (Delgado et al., 1982; Ubeda et al., 1983) and skeletal variations in rodents when applied prenatally (Huuskonen et al., 1998, 1993). Considering the period of rapid growth displayed by the central nervous system (CNS) during embryonic and early postnatal periods, some have speculated that the developing brain might also show a particular sensitivity to the effects of magnetic fields, even those of weak intensities (Adey, 1993).

Although minimal or nonsignificant teratogenic effects on the CNS have been reported (Hoyer et al., 2012; Wiley et al., 1992; Yu et al., 1993), it appears that the specific parameters of the applied field (i.e., waveform) may be one of the most important factors to consider in the study of teratogenesis. For instance, sinusoidal or square-wave pulses can be harmful to CNS growth and development but typically require field strengths on the order of a millitesla or higher to produce reliable effects (Gona et al., 1993; Juutilainen et al., 1986; Lahijani et al., 2011; Sikov et al., 1984; Ubeda et al., 1983). In contrast, we have found that wave-form patterns whose intrinsic shapes (often asymmetric or irregular pulse patterns) and interstimulus intervals have been designed to simulate common physiological or naturalistic phenomena can also be effective in altering the development of the rat brain even when applied at flux densities several magnitudes lower (<1 μT) (McKay and Persinger, 1999; McKay et al., 2003; St-Pierre et al., 2008; St-Pierre and Persinger, 2003, 2008; Whissell et al., 2009). In one study, St-Pierre et al. (2008) reported that continuous prenatal exposure to a complex composite magnetic field containing 50 sequential 200 ms intervals of specific patterns produced non-linear, intensity-dependent elevations in adult serum aminotransaminase, glucose, and uric acid. The effect was greatest for those rats that had been exposed prenatally to 30–50 nT (the range within the exposure areas) intensities compared to those exposed to the 5–20 nT or 590 nT–1.2 μT fields. Each of the patterns that comprised the "composite" field had been shown to be physiologically-effective

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http://dx.doi.org/10.1016/j.ijdevneu.2012.07.005

when presented repeatedly in other experiments involving either humans or rats and the intensities of the fields were well within the range of routine human exposure. The term "physiologically-patterned" have been applied to the components of these fields because they are more typical of the temporal structures of neuronal activity rather than sine-waves or square-waves.

In addition, we have also observed persistent behavioral changes, including diminished locomotor activity in a novel open field (Persinger, 1969; Persinger and Ossenkopp, 1973), alterations in operant conditioning (Persinger and Pear, 1972; Salzinger et al., 1990), and impaired radial maze learning (McKay et al., 2003) following prenatal exposure to low-intensity magnetic fields. These behavioral findings are consistent with the types of deficits frequently reported after insults or damage to the hippocampus (Zola-Morgan and Squire, 1993)—a region instrumental in the acquisition and recall of new information. Importantly, the growth of embryonic hippocampal neurons in vitro is also sensitive to applied electric fields (Davenport and McCaig, 1993). As a result, one may predict that very weak complex patterns of magnetic fields could have the capacity to interfere with neuronal growth and development of the hippocampus.

In this study, we set out to investigate if exposure to a weak magnetic field pattern would result in anomalies in hippocampal development and produce impairments hippocampal-dependent behavior that persist into adulthood. A general observation in physiology is that organismic responses habituate to a fixed pattern of stimulation (Persinger, 1979). Even though the development between conception and birth might be considered itself dynamic and not likely to habituate, we reasoned that by changing the pattern of the continuously presented magnetic field every day, the likelihood of habituation would be minimal. In this experiment we selected three patterns. The first was a composite pattern that produced microscopically visible qualitative anomalies in previous studies described above (St-Pierre et al., 2008). The second was a repeated pattern known to evoke LTP in hippocampal slices when applied as electric pulses. The third pattern was a variant of 50 Hz where the shape was non-sinusoidal. In addition, rather than using homogeneous intensities of the magnetic fields, we exposed the subjects to four separate ranges of intensities between 5 and 1200 nT. Although the application of more homogeneous magnetic fields have specific advantages to explore traditional EMF mechanisms, the natural environment is heterogeneous. As such, the specific ranges of intensities or flux densities were selected on the bases of their effectiveness in producing behavioral changes in adult rats and their prominence within human habitats. Our findings highlight an important sensitivity of the hippocampus to the application of weak, complex magnetic fields between 30 and 50 nT during foetal development.

2. Materials and methods

All animals were treated in accordance with the Canadian Council for Animal Care and efforts were made to minimize the number of animals used in these experiments.

2.1. Exposure apparatus and wave forms

In two separate experiments, pregnant Wistar albino female rats ($n = 2$) were housed in one of four equally sized compartments within the same transparent plastic cage (140 cm × 33 cm × 33 cm). At each end of the cage were two identical Helmholtz coils. The coils were created by wrapping cartons (38 cm × 33 cm × 27 cm) with 305 m of 30 AWG (Belden #9978) wire in a single layer (18 cm wide). To insure that the effects were due to the applied magnetic fields rather than peculiarities of one coil or the space in which the rats had been maintained, the activated coil was counterbalanced between experiments.

The temporal structures of the complex magnetic fields used in this study (composite, LTP-like, and non-sinusoidal 50 Hz) have shown empirical effectiveness in influencing brain development and behavior when individually applied (Persinger and Koren, 2007; St-Pierre et al., 2008; St-Pierre and Persinger, 2003, 2008; Whissell et al., 2009). In summary, the composite pattern was composed of 40, 250 ms signatures that involved various frequency-modulated patterns as well as those derived from mathematical relationships (Persinger, 2010; Persinger and Koren, 2007). The actual shapes of these patterns as well as their sequence can been seen in St-Pierre et al. (2008). The rationale for different temporal patterns presented in successive increments was based upon the assumption that there are electromagnetic analogues to different functional consequences of different amino-acid (spatial) sequences in polypeptides and proteins and the success of Cosic (1994) theory and application of resonance interaction between the electron-ion interactions of serial amino acids in order to predict molecular function (Wu and Persinger, 2011). The long-term potentiation-pattern pulse was repeated sequences (of positive polarity) of a primer pulse followed 170 ms later by four working pulses. This was the same timing previously shown to be effective in producing long-term potentiation in hippocampal slices when induced by electric current (Diamond et al., 1988). The 50 Hz pattern was designed from theoretical assumptions (Persinger and Koren, 2007; Whissell et al., 2009) that considered the importance of increments of 20 ms. For example the replication forks in DNA are estimated to move at about 50 nucleotides s^{-1}, that is 20 ms per nucleotide. The 50 Hz pattern was designed with a rise time and fall time of 1 ms and a plateau and trough of 20 ms.

The complex magnetic fields were generated by converting columns of numbers (between 0 and 255) from a few hundred to several thousand into voltages (between −5 and +5 V, 127 = 0 V) through a customized digital-to-analogue converter (DAC). The specific value of each successive point (0–255) determined the voltage and hence the current and magnetic field strength through the coil created the temporal pattern. The point durations (the time the voltage specified by a given number was maintained) was 1 ms and the output from a Zenith XT computer was sent to one of two coils at opposite ends of the plastic cage that contained the four compartments (and exposure intensities). All patterns were presented in a repeating cycle for 10 s followed by 40 s of no field for 24 h before the next pattern was initiated and ran for 24 h for all exposure intensities. Hence the daily patterns were composite, LTP-like, and 50 Hz before the cycle would be repeated every 4 days through gestation.

Before application of the magnetic fields, 1 male rat was placed with the two primiparous females for a total of 5 days after which the 4 males were removed. We considered that this period of time would be sufficient for the occurrence of one estrous cycle required impregnation. Following impregnation, pregnant females were exposed daily to one of three field pulses described above for 24 h per day throughout the entire 21-day gestation period. The field intensity of the complex magnetic fields within the four chambers decreased as a function of the activated coil. The four successive ranges of magnetic field intensities within the exposure areas of the compartments in which the females were housed, from either of the two coils when they were activated were: 5–20 nT (12 nT ± 2.4 nT, reference condition), 30–50 nT (40 nT ± 3.2 nT, low-intensity condition), 90–580 nT (335 nT ± 78.4 nT, medium-intensity condition), and 590 nT–1.20 μT (895 nT ± 97.6 nT, high-intensity condition). The ranges in intensities within each condition reflected the decrease of the field intensity over the distance occupied by the exposure space. These measurements were completed with a Metex 3800 multimeter and magnetic sensor probe (Electric Field Measurements, Stockbridge, MA.).

The background static geomagnetic field remained the same within the fixed spatial positions during the exposures and there were no major geomagnetic storms during the exposure periods. Even though geomagnetic activity can exceed 10 nT and increase to several hundreds of nanotesla during such periods, the frequencies are within the mHz range. During the exposure periods care was taken to insure there were no electronic devices operating in the adjacent room since magnetic fields easily penetrate block walls.

The only time the pregnant females were not exposed to the field pattern was during cage cleaning which occurred once every four days for 5–10 min. During this period, corn-cob bedding was refreshed. Water and Purina rat chow were available ad libitum and the room temperature was set between 20 and 22 °C. All 16 mothers delivered litters ranging from 9 to 15 pups. Two to three days later, the litters and their mothers were removed from the experimental setting and maintained in standard plastic housing cages in another room. Between 21 and 22 days of age, the litters were sexed and housed separately in standard wire cages. Testing began one year later and only male rats ($n = 6–7$ per condition) were used in the experiments described below.

2.2. Open field testing

The open-field was a custom-constructed wooden square box (60 cm × 60 cm × 30 cm high) that was subdivided into 25 approximately equal squares by black lines. Each rat was placed into the center region of the open field box and several open-field related measures (e.g., frequency of locomotor activity, grooming, rearing, urination, defection) were scored for a 2 min observational period. Spontaneous locomotive behavior was defined as the number of squares crossed by both hind legs during the observational period. Between subjects, the open field was cleaned using a diluted 10% ethanol cleaning solution.

2.3. Contextual fear conditioning

The fear conditioning chambers and procedure has been described in detail elsewhere (McKay et al., 2002). Briefly, rats were placed in a standard operant chamber and allowed 3 min to acclimate to the context. During this baseline period, locomotor activity, which was defined as a forward movement across the chamber

midline, was recorded. Following this, three unsignaled footshocks were delivered through a metal grid floor (2 s, 0.5 mA, 60 s intershock intervals). Sixty seconds following the last footshock, the subject was carefully removed and returned to their home cage. Twenty-four hours later, an 8 min extinction test was performed and defensive freezing was scored. Freezing was defined as a minimization of all ongoing movements except for movement of the musculature required for respiration (Bolles, 1970). Measurements were time-sampled and taken every 8 s during the entire 8-min extinction test for a total of 60 observations. These freezing measures were converted to a percentage of total observations. The conditioning chamber was cleaned between subjects with a 0.4% acetic acid solution to minimize olfactory cues.

2.4. Cell counting and hippocampal geometric analysis

A subset of rats from each treatment condition ($n = 4$) was chosen for further histological analysis. After euthanizing the subject, the brain was removed within 6 min and post-fixed in an ethanol-acetic acid-formalin solution. Post-fixed brains were then dehydrated in ethanol, defatted in chloroform, and embedded in paraffin. Care was taken to ensure that all rat brains were oriented in the same plane during embedding in order to minimize differences during sectioning. The brains were sectioned at 10 μm by a rotatory microtome and stained with toluidine blue according to standard procedures.

Examination of brain sections was completed by light microscopy at 40, 100, and 400× magnification by an investigator blind to all treatment conditions and the average number of neurons within a 6×6 grid (.061 mm^2) was counted for the various hippocampal subfields across several sections. To examine if exposure to complex magnetic field pattern adversely affected hippocampal morphological development, digitized images (8-bit) of hippocampal sections were collected and processed using image analysis software (ImageJ, NIH) (see Fig. 1B). The length of the hippocampus was measured as the maximum distance from the apex of CA3 to the most medial segment of CA1, and the width measured across the extent of the granule cell layer from each of the blades of the dentate gyrus and across CA1 to the alveus. The width of the dentate gyrus was also measured as the distance between the ends of the granule cell body.

Because in previous studies (St-Pierre et al., 2008; St-Pierre and Persinger, 2003, 2008), we found no significant differences in anomalies of the hippocampal formation or any of approximately 20 serum blood measures between the 5 and 20 nT exposed group and rats exposed to the same chambers without any active fields or age-matched colony controls, we considered that this group could serve as an appropriate within-apparatus "control" condition. Therefore, we referred to the 5–20 nT measure as a reference intensity in our study. To ensure that such a procedure was valid, we compared this group to age-matched colony controls ($n = 5$) not exposed at any time to the field conditions. We did not find statistically significant differences on any of the histological or behavioral measures between these two groups (Supplementary Fig. 1).

2.5. Statistical analyses

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS v 20.0). Group differences were assessed using one-way analysis of variance (ANOVA) and follow-up post hoc tests (Tukey's HSD). The level for statistical significance was set at $P < .05$.

3. Results

3.1. Effect of prenatal magnetic field exposure on hippocampal geometry and cell number

Our examination of Nissl-stained coronal sections taken from 1 year adult rats that were prenatally exposed to a complex sequence magnetic field revealed several interesting cytological anomalies within the hippocampus. The most extreme examples were found in the brains of rats that had been exposed to field intensities between 30 and 50 nT (Fig. 1A). Although the cytoarchitectural and laminar boundaries of the hippocampal subfields appeared well-defined and conserved across treatment conditions, prenatal exposure to the low-intensity field (30–50 nT) resulted in a reduction in the overall length [$F(3,12) = 12.2$, $P < .001$, Fig. 1C] and width of the hippocampus [$F(3,12) = 11.4$, $P < .001$, Fig. 1D]. Such abnormalities were present at all levels of the hippocampus along the septotemporal axis. Interestingly, the rats exposed to either the medium to high intensities (range 90 nT–1.2 μT) or to the reference intensity (range 5–20 nT) fields did not show this change in hippocampus size (Fig. 1C and D). Upon closer examination, there also appeared to be a decrease in CA1 pyramidal cell layer length ($P < .05$). CA3 cells were also slightly dispersed particularly within the CA3c (i.e., the part of CA3 that inserts within the dentate gyrus) and CA4 regions. However, these morphological anomalies were absent in rats exposed to the complex field pattern at flux densities above 50 nT or below 20 nT. Further examination indicated that there was no change in either dentate gyrus width ($P > .43$) or dentate granule cell layer dispersion ($P > .67$) for any of the groups (Fig. 1E).

Consistent with the observations presented above, cell counting procedures revealed a significant difference in the total number of pyramidal neurons in the CA1 and CA3 subfields as a function of field intensity [$F(3,12) = 6.23$, $P < .01$]. Follow-up post hoc analyses showed that rats prenatally exposed to the 30–50 nT field intensity showed a reduction in the number of pyramidal cells in these hippocampal subfields compared to rats exposed to higher or lower flux densities [All P's $< .05$, Fig. 2]. Importantly, the rats exposed to flux densities below 20 nT or above 50 nT were not significantly different from other, and no other significant differences emerged from the analysis of total neuronal or glial cell counts for the dentate gyrus. Finally, the ratio of cell counts to overall volume of each hippocampal region was also calculated. This normalization procedure confirmed our earlier findings that rats prenatally exposed to the low-intensity fields showed a significant reduction in hippocampal pyramidal neurons in the CA1 and CA3 subfields [F's > 12.1, P's $< .001$].

3.2. Effect of prenatal magnetic field exposure on behavior

The findings presented above suggest that application of a complex magnetic field within a very narrow intensity band throughout the prenatal period can disrupt normal hippocampal development. Therefore, we were interested in determining if there were any adverse behavioral or cognitive outcomes associated with early prenatal exposures. Because hippocampal lesions are well known to increase locomotor behavior and hyperactivity of rodents in a novel environment (Blanchard et al., 1977; Douglas and Isaacson, 1964; Teitelbaum and Milner, 1963), we first examined whether early prenatal field exposures affect behavior in a novel open field. In these experiments, rats were tested 1-year after their birth. Individual analyses of open-field related behaviors, such as locomotor activity, grooming frequency, rearing, urination, and defection revealed no significant differences between any of the treatment conditions [All F's < 1.00, P's $> .75$] (Data not shown).

Both clinical and experimental studies have also shown that damage to the hippocampus and related limbic structures can impair the ability to form new memories (Squire and Zola-Morgan, 1991). One particularly sensitive measure of hippocampal-dependent learning is contextual fear conditioning, which requires subjects to form an associative link between an aversive stimulus (i.e., a mild footshock) and the context in which conditioning occurred. Using this paradigm, we found that exposure to a complex magnetic field pattern throughout prenatal development results in a significant reduction in subsequent contextual fear conditioning [$F(3,22) = 7.37$, $P < .001$, Fig. 3]. Post hoc analyses revealed that compared to all other groups, rats previously exposed to the low-intensity condition (30–50 nT) showed a decrease in the amount of freezing during an 8 min extinction test [All P's $< .004$]. There was no differences between groups in either the frequency of midline chamber cross-overs [$P = .42$] or the amount of postshock freezing [$P = .67$] displayed during conditioning indicating that general ambulation and responses to painful stimuli were intact (Data not shown).

4. Discussion

The results of the present study demonstrate that prenatal exposure to complex patterned magnetic fields designed to impact

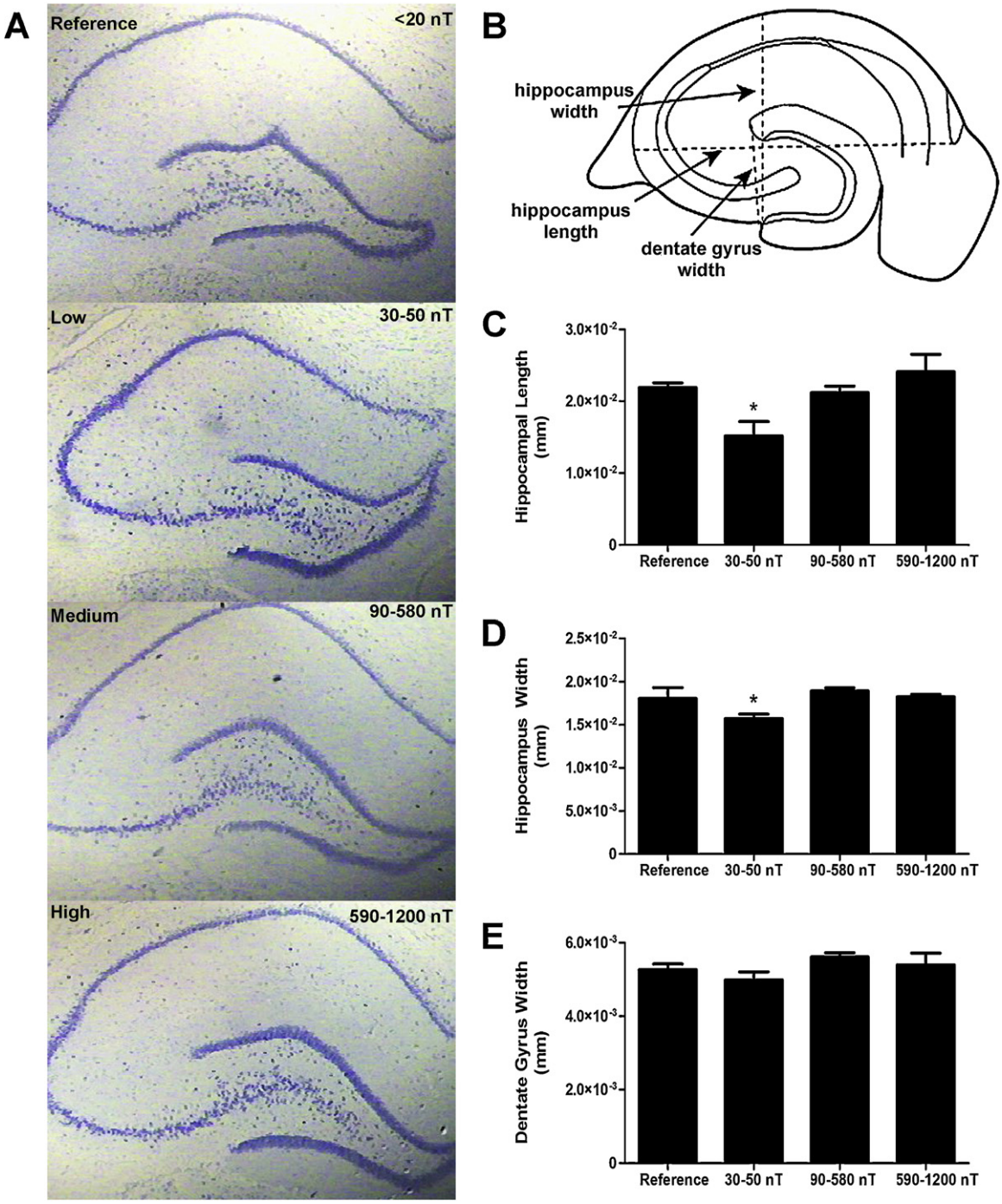

Fig. 1. (A) Photomicrographs (40× magnification) of the hippocampus of adult rats exposed throughout prenatal development to the complex magnetic field pattern (Reference: 5–20 nT, low: 30–50 nT, medium: 90–580 nT, high: 590–1200 nT). (B) Schematic of a typical measurement of hippocampal width, length and dentate gyrus width. (C–E) Average measure of hippocampal width and length in hippocampal sections, and average width of the dentate gyrus. * indicates a significant difference from the reference condition ($P < .05$).

cellular growth and migration can induce morphological changes in the hippocampus and produce impairments in hippocampal-dependent learning at a very narrow intensity window between 30 and 50 nT. Importantly, these morphological and cognitive changes were observed in 1 year old rats suggesting that the consequence of prenatal exposure to magnetic fields on hippocampal development and behavior persists well into postnatal periods. In contrast, exposure to the identical field patterns at lower (<20 nT) or higher flux densities (50–1200 nT) did not produce any obvious change in hippocampal morphology or behavior. Although we cannot rule out the possibility of more subtle effects with exposure to fields of higher intensities, we have previously shown similar cytological anomalies following application of other pulsed ("temporally asymmetric") magnetic fields of a similar intensity during prenatal development. Consistent with these findings, the application of simple invariant ("temporally symmetric") field patterns, such as sinusoidal or rectangular pulses, are generally ineffective in altering prenatal development unless much higher intensities are used. This important distinction between the waveform pattern and field strength could account for some of the contradictory evidence

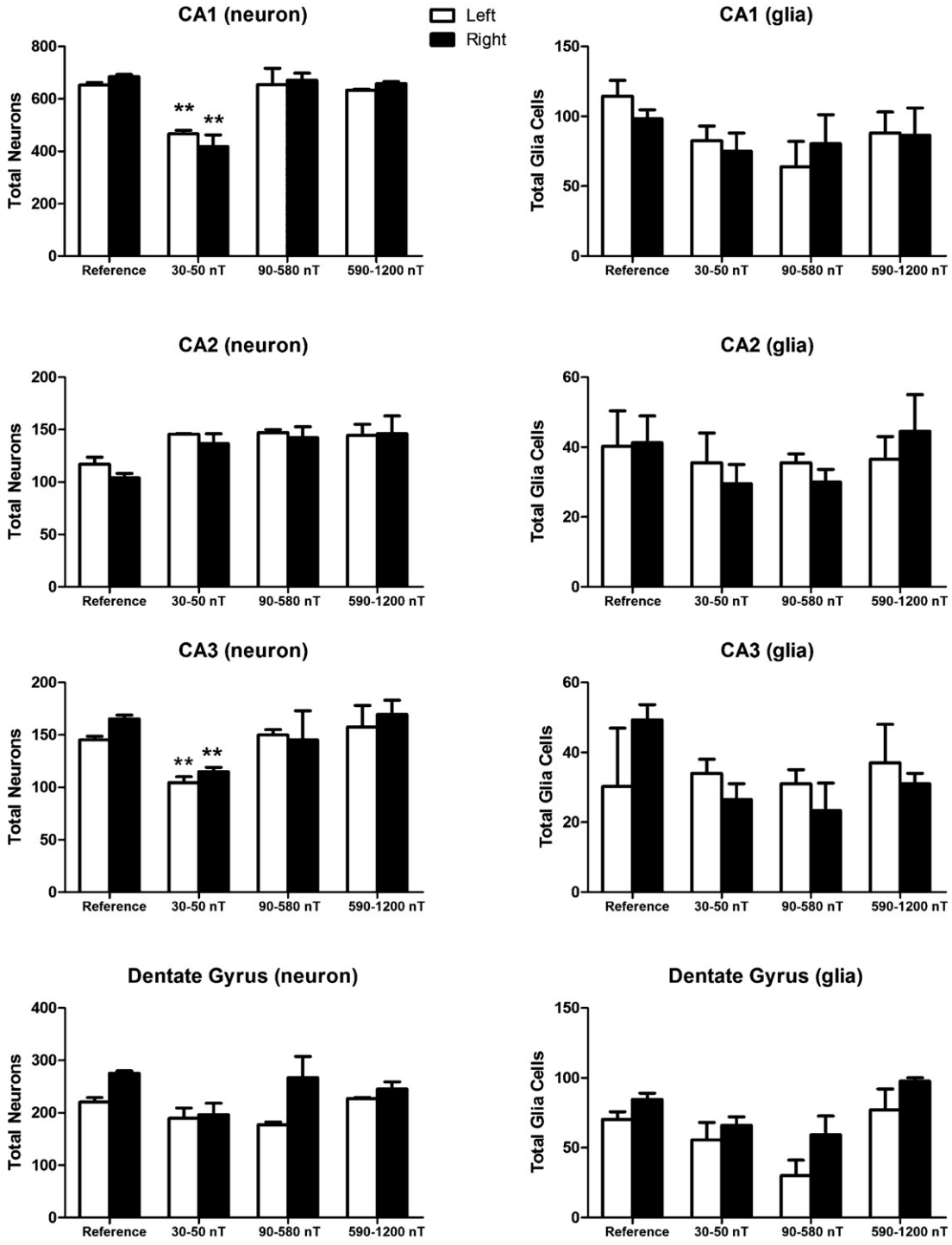

Fig. 2. Number of neuron or glial cells for each of the hippocampal subfields (CA1–CA3) and dentate gyrus of adult rats prenatally exposed to the complex magnetic field pattern of various intensities (Reference: 5–20 nT, low: 30–50 nT, medium: 90–580 nT, high: 590–1200 nT). ** indicates a significant difference from the reference condition ($P < .01$).

presented in the literature concerning the teratogenic effects of magnetic field exposure on foetal CNS development (Juutilainen et al., 1986; Ubeda et al., 1983; Wiley et al., 1992; Yu et al., 1993).

Hippocampal development begins prenatally, roughly around day 11 of gestation, and continues postnatally for up to 4 weeks in some hippocampal regions (Altman and Bayer, 1990; Bayer, 1980; Insausti et al., 2010). This accelerated and protracted period of development characteristic of the hippocampus also increases this regions vulnerability to various environmental insults and teratogenic toxins (De Santis et al., 2007; Grimm, 1987). Therefore, it is likely that even subtle deviations in the timing of some cellular and molecular events important in normal cytogenesis can result in pervasive cytoarchitectonic changes in this brain region. In support of this view, we found that exposure to low-intensity weak complex magnetic fields throughout gestation results in subtle changes in hippocampal morphogenesis. In particular, we found a reduction in hippocampal length and width along with a concomitant decrease in both CA1 and CA3 hippocampal cell number after prenatal exposure to field intensities between 30 and 50 nT.

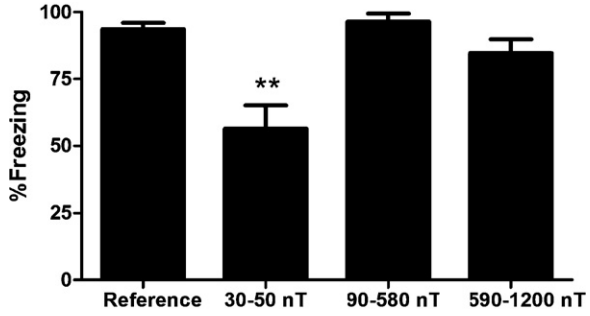

Fig. 3. Effect of prenatal exposure to a complex magnetic field on mean percentage conditioned freezing responses made during an 8 min extinction test in adult rats. Testing was conducted 24 h after contextual fear conditioning. ** indicates a significant difference from the reference condition ($P < .01$).

In rat hippocampus, the continued neurogenesis, extensive remodeling of synaptic contacts, and peak velocities of myelination also results in an exponential increase in the size of this brain region during the first four weeks of life (Altman and Bayer, 1990; Bayer, 1980). Thus, it is possible that the loss of hippocampal neurons we observed following low-intensity prenatal exposures may have occurred during postnatal periods of competitive cell elimination. This possibility seems unlikely since exposure to these fields terminated shortly after birth and the majority of hippocampal pyramidal neurons are formed during embryonic day 14/15. In contrast, most dentate granule cells (~80%) are generated within first four weeks of birth (Bayer and Altman, 1974). However, we could not find any evidence that our field exposures adversely affected dentate granule cell number or granule cell layer development. Another possibility is that exposure to complex magnetic field patterns, especially those of low-intensity, might interfere with the proliferating capacity of progenitor cells during key periods of early hippocampal cytogenesis. Indeed, recent work has shown that in vivo exposure to pulsed magnetic fields of this intensity can interfere with normal growth signaling pathways in B16-BL6 melanoma cells resulting in diminished mitotic activity of these cells (Hu et al., 2010). We are presently examining whether the low-intensity fields used in our experiments might influence the regulation of cell cycle signaling factors that ultimately impact mitotic progression of neuroblasts.Given the prominent role that the hippocampus plays in the formation of memory during fear conditioning, we anticipated that the ability to acquire or retain fear-related memories might be impacted by prenatal exposure to the complex magnetic field. Consistent with this idea, we found that rats exposed to the low-intensity complex magnetic field pattern showed statistically significant impairments in consolidation of contextual fear memory. In contrast, prenatal exposure to the same field pattern at flux densities either below 20 nT or above 50 nT did not affect fear learning as adults. These findings extend on earlier work showing that adult rats exposed to a complex magnetic field throughout the entire prenatal period exhibit deficits on a range of learning/memory measures, including spatial learning and conditioned taste aversion (McKay et al., 2003; St-Pierre and Persinger, 2008). Finally, it should be noted that prenatally exposed rats do not differ from sham-exposed rats in terms of body mass, physical appearance, activity level, or incidence of disease suggesting that the observed behavioral changes that follow the application of these fields during prenatal periods reflect subtle changes in the microstructural organization of the hippocampus and other related structures.

Until recently, the effects of magnetic fields on biological systems have generally involved mechanisms involving some variant of Faraday's principal of induction (e.g., that a changing magnetic field is associated with a changing electric field). However, the flow of current necessary to achieve such effects requires flux densities within the millitesla to tesla range. As such, the bioeffects of the weak (<1 μT) magnetic fields employed in our study are unlikely to involve current induction through classical mechanisms. Nonetheless, weak magnetic fields have been reported to influence a number of important biological or electrochemical processes—many of which play an important role in organogenesis, including development of the brain. These include altering RNA, DNA, and protein synthesis, ion transport across membranes, voltage-sensitive channels, calcium-dependent signaling pathways, the structure of the phospholipid bilayer within cell membranes, or the binding of ligands to receptors at the cell surface (Conti et al., 1985; Hendee and Boteler, 1994; McCann et al., 1998; Persinger et al., 1973; Phillips et al., 1992; Ruiz-Gomez and Martinez-Morillo, 2009; Ubeda et al., 1994). An interesting feature shared by many of these studies, including our own, is that certain combinations of field strengths and frequencies are more effective than others in producing biological effects. These findings suggest the effects of weak magnetic fields can be amplified through the presence of optimal "windows" of frequency and intensity in which resonant interactions may occur within biological tissues (i.e., through altering the vibration or rotational energy of surrounding ionic species or biomolecules). The introduction of such intensity widows indicates that exposure to an appropriately "tuned" magnetic fields could potentially influence biological function at energies significantly lower than those required for non-tuned fields. Indeed, resonant interactions have been shown to produce effects as much as five orders of magnitude greater than those achieved through classical Faraday mechanisms (Adey, 1993). In further support of this idea, we have observed that rats exposed prenatally to the 30–50 nT complex magnetic field pattern (e.g., composite field used in this study) show significantly more anomalies (e.g., ectopic cell migration) in the hippocampal formation compared to rats exposed to stronger or weaker intensities, including colony room controls (St-Pierre and Persinger, 2003). In contrast, exposure to the same intensity of a frequency-modulated field, that when presented for 30 min to adult rats evokes analgesia comparable to 4 mg/kg of morphine (Martin et al., 2004), did not show hippocampal anomalies when applied prenatally indicating that the specificity of the biological effect of a magnetic field is associated with an optimal intensity applied with a specific pattern rather than exposure to just any intensity-matched field.

In conclusion, continuous prenatal exposure to a weak complex magnetic field can result in subtle but lasting changes in hippocampal cytogenesis and behavior. Importantly, the effective intensities or flux densities of the fields employed in our study was between 20 and 1200 nT (or 0.2–12 mG), which is well within the range of routine human exposure. Given the ubiquity of electrical appliances that are sources of magnetic field emission in the home and environment, the potential for interaction or interference between various high frequency magnetic fields to produce "pulses" whose intensities and temporal patterns are similar to that of the human brain becomes increasingly likely. Depending on the timing of exposure and the composition of the emergent complex magnetic fields generated through these interactions, there may be the potential for adverse effects on CNS growth and development. Although the resultant effects would likely be subtle, there may be significant long-lasting consequences on the organism and its behavior.

Acknowledgments

N.M.F. is a James Hudson Brown-Alexander Brown Coxe postdoctoral fellow and was supported from fellowships provided by the Natural Sciences and Engineering Council of Canada and Canadian Institutes of Health Research Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijdevneu.2012.07.005.

References

Adey, W.R., 1993. Biological effects of electromagnetic fields. Journal of Cellular Biochemistry 51, 410–416.

Altman, J., Bayer, S.A., 1990. Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. Journal of Comparative Neurology 301, 365–381.

Bayer, S.A., 1980. Development of the hippocampal region in the rat. II. Morphogenesis during embryonic and early postnatal life. Journal of Comparative Neurology 190, 115–134.

Bayer, S.A., Altman, J., 1974. Hippocampal development in the rat: cytogenesis and morphogenesis examined with autoradiography and low-level X-irradiation. Journal of Comparative Neurology 158, 55–79.

Blanchard, D.C., Blanchard, R.J., Lee, M.C., Fukunaga, K.K., 1977. Movement arrest and the hippocampus. Physiological Psychology 5, 331–335.

Bolles, R.C., 1970. Species-specific defense reactions and avoidance learning. Psychological Review 77, 32–48.

Conti, P., Gigante, G.E., Alesse, E., Cifone, M.G., Fieschi, C., Reale, M., Angeletti, P.U., 1985. A role for Ca2+ in the effect of very low frequency electromagnetic field on the blastogenesis of human lymphocytes. FEBS Letters 181, 28–32.

Cosic, I., 1994. Macromolecular bioactivity: is it resonant interaction between macromolecules?––Theory and applications. IEEE Transactions on Biomedical Engineering 41, 1101–1114.

Davenport, R.W., McCaig, C.D., 1993. Hippocampal growth cone responses to focally applied electric fields. Journal of Neurobiology 24, 89–100.

De Santis, M., Cesari, E., Nobili, E., Straface, G., Cavaliere, A.F., Caruso, A., 2007. Radiation effects on development. Birth Defects Research Part C: Embryo Today 81, 177–182.

Delgado, J.M., Leal, J., Monteagudo, J.L., Gracia, M.G., 1982. Embryological changes induced by weak, extremely low frequency electromagnetic fields. Journal of Anatomy 134, 533–551.

Diamond, D.M., Dunwiddie, T.V., Rose, G.M., 1988. Characteristics of hippocampal primed burst potentiation in vitro and in the awake rat. Journal of Neuroscience 8, 4079–4088.

Douglas, R.J., Isaacson, R.L., 1964. Hippocampal lesions and activity. Psychonomic Science 1, 187–188.

Gona, A.G., Yu, M.C., Gona, O., al-Rabiai, S., Von Hagen, S., Cohen, E., 1993. Effects of 60 Hz electric and magnetic fields on the development of the rat cerebellum. Bioelectromagnetics 14, 433–447.

Grimm, V.E., 1987. Effect of teratogenic exposure on the developing brain: research strategies and possible mechanisms. Developmental Pharmacology and Therapeutics 10, 328–345.

Hendee, W.R., Boteler, J.C., 1994. The question of health effects from exposure to electromagnetic fields. Health Physics 66, 127–136.

Hoyer, C., Vogt, M.A., Richter, S.H., Zaun, G., Zahedi, Y., Maderwald, S., Ladd, M.E., Winterhager, E., Grummer, R., Gass, P., 2012. Repetitive exposure to a 7 Tesla static magnetic field of mice in utero does not cause alterations in basal emotional and cognitive behavior in adulthood. Reproductive Toxicology 34, 86–92.

Hu, J.H., St-Pierre, L.S., Buckner, C.A., Lafrenie, R.M., Persinger, M.A., 2010. Growth of injected melanoma cells is suppressed by whole body exposure to specific spatial-temporal configurations of weak intensity magnetic fields. International Journal of Radiation Biology 86, 79–88.

Huuskonen, H., Juutilainen, J., Julkunen, A., Maki-Paakkanen, J., Komulainen, H., 1998. Effects of low-frequency magnetic fields on fetal development in CBA/Ca mice. Bioelectromagnetics 19, 477–485.

Huuskonen, H., Juutilainen, J., Komulainen, H., 1993. Effects of low-frequency magnetic fields on fetal development in rats. Bioelectromagnetics 14, 205–213.

Insausti, R., Cebada-Sanchez, S., Marcos, P., 2010. Postnatal development of the human hippocampal formation. Advances in Anatomy, Embryology and Cell Biology 206, 1–86.

Juutilainen, J., 2005. Developmental effects of electromagnetic fields. Bioelectromagnetics Suppl. 7, S107–S115.

Juutilainen, J., Harri, M., Saali, K., Lahtinen, T., 1986. Effects of 100 Hz magnetic fields with various waveforms on the development of chick embryos. Radiation and Environmental Biophysics 25, 65–74.

Lahijani, M.S., Bigdeli, M.R., Kalantary, S., 2011. Effects of sinusoidal electromagnetic fields on histopathology and structures of brains of preincubated white Leghorn chicken embryos. Electromagnetic Biology and Medicine 30, 146–157.

Lovely, R.H., 1988. Recent studies in the behavioral toxicology of ELF electric and magnetic fields. Progress in Clinical and Biological Research 257, 327–347.

Martin, L.J., Koren, S.A., Persinger, M.A., 2004. Thermal analgesic effects from weak, complex magnetic fields and pharmacological interactions. Pharmacology Biochemistry and Behavior 78, 217–227.

McCann, J., Dietrich, F., Rafferty, C., 1998. The genotoxic potential of electric and magnetic fields: an update. Mutation Research 411, 45–86.

McKay, B.E., Lado, W.E., Martin, L.J., Galic, M.A., Fournier, N.M., 2002. Learning and memory in agmatine-treated rats. Pharmacology Biochemistry and Behavior 72, 551–557.

McKay, B.E., Persinger, M.A., 1999. Geophysical variables and behavior: LXXXVII. Effects of synthetic and natural geomagnetic patterns on maze learning. Perceptual and Motor Skills 89, 1023–1024.

McKay, B.E., St-Pierre, L.S., Persinger, M.A., 2003. Radial maze proficiency of adult Wistar rats given prenatal complex magnetic field treatments. Developmental Psychobiology 42, 1–8.

Mcleod, B.R., Liboff, A.R., Smith, S.D., 1992. Biological-systems in transition – sensitivity to extremely low-frequency fields. Electro- and Magnetobiology 11, 29–42.

Nordeon, B., Ramel, C., 1992. Interaction Mechanism of Low-Level Electromagnetic Fields in Living Systems. Oxford University Press, Oxford.

Persinger, M.A., 1969. Open-field behavior in rats exposed prenatally to a low intensity-low frequency, rotating magnetic field. Developmental Psychobiology 2, 168–171.

Persinger, M.A., 1979. A first order approximation of satiation time: (IRT)2/Rt. Perceptual and Motor Skills 49, 649–650.

Persinger, M.A., 2010. 10–20 Joules as a neuromolecular quantum in medicinal chemistry: an alternative approach to myriad molecular pathways? Current Medicinal Chemistry 17, 3094–3098.

Persinger, M.A., Koren, S.A., 2007. A theory of neurophysics and quantum neuroscience: implications for brain function and the limits of consciousness. International Journal of Neuroscience 117, 157–175.

Persinger, M.A., Ludwig, H.W., Ossenkopp, K.P., 1973. Psychophysiological effects of extremely low frequency electromagnetic fields: a review. Perceptual and Motor Skills 36, 1139–1151.

Persinger, M.A., Ossenkopp, K.P., 1973. Some behavioral effects of pre-and neo-natal exposure to an ELF rotating magnetic field. International Journal of Biometeorology 17, 217–220.

Persinger, M.A., Pear, J.J., 1972. Prenatal exposure to an ELF-rotating magnetic field and subsequent increase in conditioned suppression. Developmental Psychobiology 5, 269–274.

Phillips, J.L., Haggren, W., Thomas, W.J., Ishida-Jones, T., Adey, W.R., 1992. Magnetic field-induced changes in specific gene transcription. Biochimica et Biophysica Acta 1132, 140–144.

Ruiz-Gomez, M.J., Martinez-Morillo, M., 2009. Electromagnetic fields and the induction of DNA strand breaks. Electromagnetic Biology and Medicine 28, 201–214.

Salzinger, K., Freimark, S., McCullough, M., Phillips, D., Birenbaum, L., 1990. Altered operant behavior of adult rats after perinatal exposure to a 60 Hz electromagnetic field. Bioelectromagnetics 11, 105–116.

Sikov, M.R., Montgomery, L.D., Smith, L.G., Phillips, R.D., 1984. Studies on prenatal and postnatal development in rats exposed to 60 Hz electric fields. Bioelectromagnetics 5, 101–112.

Squire, L.R., Zola-Morgan, S., 1991. The medial temporal lobe memory system. Science 253, 1380–1386.

St-Pierre, L.S., Mazzuchin, A., Persinger, M.A., 2008. Altered blood chemistry and hippocampal histomorphology in adult rats following prenatal exposure to physiologically-patterned, weak (50–500 nanoTesla range) magnetic fields. International Journal of Radiation Biology 84, 325–335.

St-Pierre, L.S., Persinger, M.A., 2003. Conspicuous histomorphological anomalies in the hippocampal formation of rats exposed prenatally to a complex sequenced magnetic field within the nanotesla range. Perceptual and Motor Skills 97, 1307–1314.

St-Pierre, L.S., Persinger, M.A., 2008. Behavioral changes in adult rats after prenatal exposures to complex, weak magnetic fields. Electromagnetic Biology and Medicine 27, 355–364.

Teitelbaum, H., Milner, P., 1963. Activity changes following partial hippocampal lesions in rats. Journal of Comparative and Physiological Psychology 56, 284–289.

Ubeda, A., Leal, J., Trillo, M.A., Jimenez, M.A., Delgado, J.M., 1983. Pulse shape of magnetic fields influences chick embryogenesis. Journal of Anatomy 137 (Pt 3), 513–536.

Ubeda, A., Trillo, M.A., Chacon, L., Blanco, M.J., Leal, J., 1994. Chick embryo development can be irreversibly altered by early exposure to weak extremely-low-frequency magnetic fields. Bioelectromagnetics 15, 385–398.

Whissell, P.D., Tsang, E.W., Mulligan, B.P., Persinger, M.A., 2009. Prenatal exposures to LTP-patterned magnetic fields: quantitative effects on specific limbic structures and acquisition of contextually conditioned fear. International Journal of Neuroscience 119, 1–14.

Wiley, M.J., Corey, P., Kavet, R., Charry, J., Harvey, S., Agnew, D., Walsh, M., 1992. The effects of continuous exposure to 20 kHz sawtooth magnetic fields on the litters of CD-1 mice. Teratology 46, 391–398.

Wu, H.P., Persinger, M.A., 2011. Increased mobility and stem-cell proliferation rate in Dugesia tigrina induced by 880 nm light emitting diode. Journal of Photochemistry and Photobiology B 102, 156–160.

Yu, M.C., Gona, A.G., Gona, O., al-Rabiai, S., Von Hagen, S., Cohen, E., 1993. Effects of 60 Hz electric and magnetic fields on maturation of the rat neopallium. Bioelectromagnetics 14, 449–458.

Zola-Morgan, S., Squire, L.R., 1993. Neuroanatomy of memory. Annual Review of Neuroscience 16, 547–563.